



PATENT #9

THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Applicant : Kaempfer et al.

Serial No. : 09/801,371

Filed : March 7, 2001

Group Art Unit: 1645

For : REGULATION OF GENE EXPRESSION THROUGH
MANIPULATION OF m RNA SPLICING AND ITS USES

AMENDMENT AND SEQUENCE LISTING SUBMISSION

I hereby certify that this paper is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231

July 18, 2001

Date of Deposit

Rochelle K. Seide

Attorney Name

Signature

32,300

PTO Registration No

July 18, 2001

Date of Signature

Assistant Commissioner for Patents

Washington, D.C. 20231

Sir:

In response to the Notice of Incomplete Reply mailed on June 29, 2001,
Applicants submit the following amendment and remarks. Applicant submit herewith an
Abstract of the technical disclosure. Applicants also submit herewith a substitute
Sequence Listing in computer and paper form in accordance with 37 C.F.R. §1.821-
1.825. Applicants also request a one month extension of time for response under 37

C.F.R. 1.136 (a) and enclose the required fee pursuant to 37 C.F.R. 1.17 (a)(1).

Applicants respectfully request consideration of the following amendments and remarks.

IN THE SPECIFICATION

Please **insert** the abstract attached hereto on a separate page captioned "SUBSTITUTE ABSTRACT."

Please **delete** the sequence listing and substitute, therefore, the attached substitute sequence listing.

Please **amend** the Brief Description of the Drawings beginning at page 11, line 23 and ending at page 12, line 4 with the following rewritten paragraph:

Figure 5

Nuclease sensitivity mapping of TNF- α 3'UTR- α EP RNA

5' End-labeled 3'UTR- α EP RNA was digested with T1, U2 or V1 nuclease directly to assay structure (*str*) (*c*, without nuclease) and, for T1 and U2, also after denaturation at 50°C in 7 M urea (*seq*). Nucleotide ladder was generated by alkaline hydrolysis (OH). Autoradiogram of sequencing gel is shown (Fig. 5A). Stem and loop regions relate to secondary structure at right, showing sites of nuclease attack, based on multiple analyses. GGGC is from plasmid. 2-APRE is the 2-AP response element (SEQ ID NO. 7) (Fig. 5B). Phylogenetic conservation of sequences is shown for *Homo sapiens* (human) (SEQ ID NO. 8), *Sus scrofa* (wild boar) (SEQ ID NO. 9),

Oryctolagus cuniculus (rabbit) (SEQ ID NO. 10), *Bos taurus* (bull) (SEQ ID NO.11) and *Capra hircus* (goat) (SEQ ID NO.12) (Fig. 5C).

Please **amend** in the Detailed Description of Preferred Embodiments the paragraph on page 15, lines 10-19 with the following paragraph:

SEQ ID NO:1, SEQ ID NO:5, SEQ ID NO:2 and SEQ ID NO:6 are shown in the following Table 1.

Table 1

SEQ ID NO:1 (upper strand) and SEQ ID NO:5 (lower strand)

```

GAATTCAAACCTGGGGCCTCCAGAACTCACTGGGGCCTACAGCTTTGATCCCTGACATCTG
2817-----+-----+-----+-----+-----+-----+2876
CTTAAGTTTGACCCCGGAGGTCTTGAGTGACCCCGGATGTCGAAACTAGGGACTGTAGAC

GAATCTGGAGACCAGGGAGCCTTTGGTTCTGGCCAGAATGCTGC
2877-----+-----+-----+-----+-----+-----2920
CTTAGACCTCTGGTCCCTCGGAAACCAAGACCGGTCTTACGACG

```

SEQ ID NO:2 (upper strand) and SEQ ID NO:6 (lower strand)

```

TCAAACCTGGGGCCTCCAGAACTCACTGGGGCCTACAGCTTTGA
2821-----+-----+-----+-----+-----+-----2863
CTTAAGTTTGACCCCGGAGGTCTTGAGTGACCCCGGATGTCGA

```

Please **amend** in the Detailed Description of Preferred Embodiments the paragraph beginning on page 23, line 26, and ending on page 24, line 9 with the following paragraph:

pTNF- α (Δ 3'UTR-i3EP) was constructed by joining SphI-digested pTNF- α (Δ 3'UTR) DNA to the 333-bp SphI-SphI TNF- β gene fragment described above which is comprised of 153 terminal bp of the 3'-UTR, the polyadenylation site, and downstream sequences. This plasmid was then digested with XhoI which cuts uniquely inside TNF- α intron 3. A 2-APRE DNA fragment abutted by XhoI restriction sites was then inserted into this site. The 2-APRE DNA fragment was obtained by polymerase chain reaction using pTNF- α DNA as template and two synthetic DNA primers of sequences 5'-CCGCTCGAGAATTCAAACCTGGGGCCTCC-3' (SEQ ID NO: 3) and 5'-CCGCTCGAGTGCAGCATTCTGGCCAGAACC-3' (SEQ ID NO:4) as 5' and 3' primers, respectively; the DNA product was digested with XhoI before ligation. Orientation of the 2-APRE insert in pTNF- α (Δ 3'UTR-i3EP) was determined by analysis of DNA fragments generated upon PvuII/PstI digestion.

REMARKS

In response to the Notice of Incomplete Reply mailed on June 29, 2001, Applicants submit the following amendment and remarks. Applicant submit herewith an Abstract of the technical disclosure. Applicants also submit herewith a substitute Sequence Listing in computer and paper form in accordance with 37 C.F.R. §1.821-

1.825. Applicants also request a one month extension of time for response under 37 C.F.R. 1.136 (a) and enclose the required fee pursuant to 37 C.F.R. 1.17 (a)(1).

A Sequence Listing in computer readable format has not previously been filed in this application. Applicants submit herewith an initial copy of the Sequence Listing in computer form and a substitute copy of the Sequence Listing in paper form.

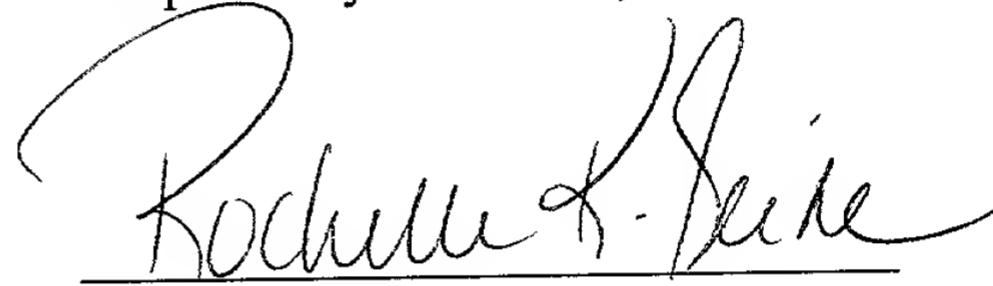
Rewritten paragraphs appear in the preceding "IN THE SPECIFICATION" section. Attached hereto is a marked-up version of the changes made to the specification paragraphs by the instant amendment. The attached page is captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE" and is only included for the Examiner's convenience. Should any discrepancies be discovered, the version presented in the preceding "IN THE SPECIFICATION" section shall take precedence.

The content of the paper and computer readable copies of the Sequence Listing submitted in accordance with 37 C.F.R. §1.821(c) and (e) are the same and do not include new matter.

PATENT

Applicants enclose the fee required for a one month extension of time in accordance with 37 C.F.R. 1.136 (a) and enclose the required fee pursuant to 37 C.F.R. 1.17 (a)(1). However, please charge any fees associated with this filing or credit any overpayment to Deposit Account No. 02-4377. Two copies of this paper are enclosed. Applicants also enclose a copy of the Notice of Incomplete Reply.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADEIN THE SPECIFICATION

The Brief Description of the Drawings beginning at page 11, line 23 and ending at page 12, line 4 has been amended as follows:

Figure 5*Nuclease sensitivity mapping of TNF- α 3'UTR- α EP RNA*

5' End-labeled 3'UTR- α EP RNA was digested with T1, U2 or V1 nuclease directly to assay structure (*str*) (*c*, without nuclease) and, for T1 and U2, also after denaturation at 50°C in 7 M urea (*seq*). Nucleotide ladder was generated by alkaline hydrolysis (OH). Autoradiogram of sequencing gel is shown (Fig. 5A). Stem and loop regions relate to secondary structure at right, showing sites of nuclease attack, based on multiple analyses. GGGC is from plasmid. 2-APRE is the 2-AP response element (SEQ ID NO. 7) (Fig. 5B). Phylogenetic conservation of sequences is shown for *Homo sapiens* (human) (SEQ ID NO. 8), *Sus scrofa* (wild boar) (SEQ ID NO. 9), *Oryctolagus cuniculus* (rabbit) (SEQ ID NO. 10), *Bos taurus* (bull) (SEQ ID NO. 11) and *Capra hircus* (goat) (SEQ ID NO. 12) (Fig. 5C).

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GAATCTGGAGACCAGGGAGCCTTTGGTTCTGGCCAGAATGCTGC
2877-----+-----+-----+-----+-----2920
CTTAGACCTCTGGTCCCTCGGAAACCAAGACCGGTCTTACGACG
```

SEQ ID NO:2 (upper strand) and SEQ ID NO:6 (lower strand)

```
TCAAACCTGGGGCCTCCAGAACTCACTGGGGCCTACAGCTTTGA
2821-----+-----+-----+-----+-----2863
CTTAAGTTTGACCCCGGAGGTCTTGAGTGACCCCGGATGTCGA
```

The following paragraph in the Detailed Description of Preferred Embodiments beginning on page 23, line 26, and ending on page 24, line 9 has been amended as follows:

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sequences. This plasmid was then digested with XhoI which cuts uniquely inside TNF- α intron 3. A 2-APRE DNA fragment abutted by XhoI restriction sites was then inserted into this site. The 2-APRE DNA fragment was obtained by polymerase chain reaction using pTNF- α DNA as template and two synthetic DNA primers of sequences 5'-CCGCTCGAGAATTCAAACCTGGGGCCTCC-3' (SEQ ID NO: 3) and 5'-CCGCTCGAGTGCAGCATTCTGGCCAGAACC-3' (SEQ ID NO:4) as 5' and 3' primers, respectively; the DNA product was digested with XhoI before ligation. Orientation of the 2-APRE insert in pTNF- α (Δ 3'UTR-i3EP) was determined by analysis of DNA fragments generated upon PvuII/PstI digestion.

SUBSTITUTE ABSTRACT

A *cis*-acting nucleotide sequence which is capable of rendering the removal of intron/s from a precursor transcript encoded by a gene, which gene harbors at least one such *cis*-acting nucleotide sequence, occurring during the production of mRNA of said gene, dependent upon activation of a *trans*-acting factor. The *trans*-acting factor is an RNA-activated protein kinase which is capable of phosphorylating the α -subunit of eukaryotic initiation factor 2, or the RNA-activated protein kinase (PKR). The *cis*-acting nucleotide sequence can be derived from the 3' untranslated region of the human tumor necrosis factor α gene (TNF- α 3'-UTR). The *cis*-acting nucleotide sequence may comprise the nucleotide sequence as denoted by SEQ ID NO:1; or biologically functional fragments, derivatives, mutants and homologues of the nucleotide sequence as denoted by SEQ ID NO:1; or a nucleotide sequence whose complementary nucleotide sequence hybridizes, under conditions which allow for such hybridization to occur, with the nucleotide sequences as denoted by SEQ ID NO:1 or biologically functional fragments, derivatives, mutants and homologues of the nucleotide sequence as denoted by SEQ ID NO:1.